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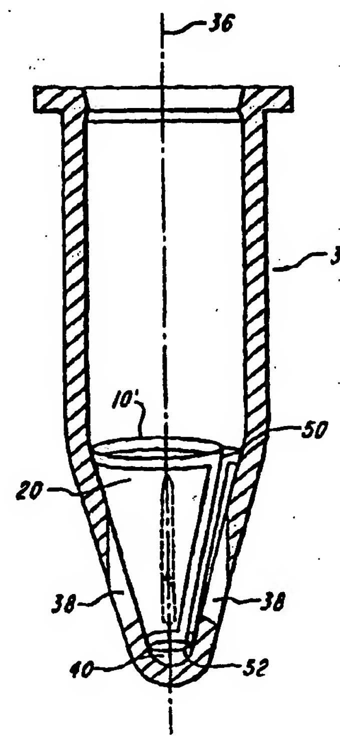
(54) Title: ULTRAFILTRATION DEVICE AND METHOD OF FORMING SAME

(57) Abstract

An ultrafiltration device has a filter membrane sealed inside a reservoir body, such as a tube. The tube has one or more ports and a closed portion distal to the port(s), and the filter membrane is sealed to the body along a closed contour widely surrounding the port(s) to provide a large area filtered outflow path. The method is effective to rapidly isolate a predetermined amount of a desired retentate in the distal portion of the tube. The method and device are also useful for quantitative transfer of smaller molecules and for multi-step processing of sample arrays. The vessels have a high filter area to volume ratio, maintain open filter surfaces and high rates of filtration throughout the spin, and are fully compatible with robotic loading, multistage operation and *in situ* multiwell plate filtrate and/or retentate assay or transfer. Attachment of the filter may be effected by heat welding. Preferably the vessel and filter are positioned between a press member and a heat sink and a super heated tool contacts the press member to selectively deliver a defined bolus of heat to the weld areas.



ULTRAFILTRATION DEVICE AND METHOD OF FORMING SAME

BACKGROUND OF THE INVENTION

Various devices are known for isolating a retentate containing a high molecular weight material, such as DNA or protein, through centrifugal ultrafiltration. The yields and amounts of retentate achieved using these techniques vary greatly due to the size, shape and position of filter membrane, the positions of outlets and/or the presence of ledges, corners or compartments in the devices.

Often these devices have associated limitations or drawbacks. For example, a device may be ineffective to prevent filtration of retentate to near dryness, or may have a design that hinders access to, or prevents complete pipette recovery of, the retentate due to chamber geometry, surface tension spreading, or the like. Also, a device may attain only a low yield or poor separation, or may require excessive centrifuge times. Additionally, a device may be poorly adapted for, or entirely incapable of, being prepared by or being used with robotic or other automated devices. Further, a technique or device may be uneconomic due, for example, to inefficient utilization of filter membrane area, and/or to manufacturing cost, and/or to requiring a long centrifuge time.

Therefore, a need exists for a centrifugal ultrafiltration device that can be dependably manufactured and used.

There is also a need for a separation technique that is rapid, effective and amenable to automated implementation.

There is also a need for improved processes for the manufacture or assembly of filtration or concentration vessels.

SUMMARY OF THE INVENTION

One or more of the foregoing ends are achieved in accordance with the present invention by providing a separation vessel having a conical region extending to a closed tip and a port in the wall of the conical region covered by a filter. The filter has a pore size and structure such that when centrifuged, fluid material such as solvent and solutes with a molecular weight below a threshold level passes through the filter and out the port.

inner surface to fuse the filter backing membrane to the vessel wall.

The invention in another aspect provides a centrifugal concentrator having an alignment structure, such as a rib, which extends in a plane through the concentrator tube axis and serves to align a wedge-shaped membrane squarely along the axis during insertion of the membrane and assures that the filter edges are located away from ports of the vessel. The filter may extend substantially the full circumference, so that the well-aligned edges abut and seal precisely when pressed in along the axial direction with a tack welder, such as a conical tip and/or slotted insertion/heat sealing tool. The tool may also melt the vessel rib over the seated butt edges. A seating ledge provided in the vessel wall to engage the top edge of the filter further aids in orienting or positioning the truncated cone filter membrane, and stabilizes filter position during handling or assembly.

In yet another aspect the concentrator tube is configured to fit into and be supported by a filtrate collection tube, and the concentrator tube has a top sealing surface with a deflectable sealing lip that seals against the cap of the filtrate collection tube. The lip deflects in response to outside pressure within the capped collection tube, opening during centrifugation to allow venting via a bypass channel so pressure may vent from the collection tube to the concentrator tube, without leaking or blowing aerosols out to the centrifuge drum. This allows the concentrator tube to be overfilled, i.e., to be loaded into a fixed angle carrier at a higher fill level such that the fluid contents wet the cap, and yet to be processed without spillover or leakage, thus increasing the attainable concentration ratio and enhancing the speed and yield of the concentration process.

The invention also contemplates a separation vessel manufactured with a clamshell construction as part of a vessel array having the form of a strip or row of two or more vessels. In accordance with this aspect of the invention, a sheet of suitable polymer material is formed with a number *n* of identically-shaped troughs, each trough corresponding to one-half of the desired chamber shape, and including one or more ports formed in a conically sloping region thereof. A sheet of filter material is then placed over the multi-trough polymer sheet, and may optionally be pressed into the troughs and sealingly attached to cover the ports. Attachment is done by advancing one or more tools, such as a press mold or a hot wire die, which advantageously may be advanced in a direction perpendicular to the plane of the sheet, avoiding shear movement at the surface

place. The vessel or press plate may be provided with protrusions or partial ribs to automatically center the heat transfer tooling in the vessel and assure complete welding of the intended weld lines in areas to seal the filter over the ports and prevent ballooning of its central region.

BRIEF DESCRIPTION OF DRAWINGS

These and other features of the invention will be understood from the discussion below and illustration of representative embodiments in the drawings, wherein:

Figure 1 shows a shaped sheet of filter membrane suitable for the vessel of the present invention;

Figure 2 shows an alternate shape for the filter membrane used in the invention;

Figures 2A and 2B illustrate cutting patterns for obtaining the membranes of Figures 1 and 2 respectively from large continuous sheets;

Figure 3 illustrates a first embodiment of a separation vessel in accordance with the invention;

Figure 4 illustrates assembly of a filter into the vessel of Figure 3;

Figure 5 illustrates another step of assembly of the vessel of Figure 3;

Figure 6 illustrates one embodiment of a heat welding inserter tool for carrying out the step of Figure 5;

Figure 7 illustrates a completed separation vessel having a square flange;

Figure 8 illustrates a strip or cartridge array of separation vessels in accordance with another embodiment;

Figure 9 illustrates another embodiment of a vessel of the invention wherein a filter membrane extends distally of the outlet port;

Figures 10A-10D illustrate steps of manufacturing and use of a strip array embodiment like that of Figure 8;

Figures 11A-11D illustrate another embodiment of a concentrator vessel and filter of the invention;

Figures 12A-12C illustrate venting operation of the embodiment of Figures 11A-11D;

may be calculated by dividing its active area by its total active area, inactive area, and cutting waste area. In the embodiment of FIG. 1, the membrane has an active area of approximately 1.0 cm^2 and an inactive area of approximately 0.22 cm^2, with no cutting waste area. Thus, the manufacturing efficiency is approximately 0.82 or 82%. This level of efficiency for the wedge-like filter membrane is much higher than the utilization efficiencies of disk-shaped membrane designs currently used in the art.

An alternate embodiment of the filter membrane 10 of FIG. 1 is shown in FIG. 2. The filter membrane 10′ depicted in FIG. 2 is also of a substantially wedge like design, however, the filter membrane of FIG. 2 has proximal and distal ends 12′, 14′ that are formed from two substantially straight edges. The sides 16′, 18′ which connect the ends 12′, 14′, however, are generally identical to the sides 16, 18 of the filter membrane of FIG. 1. The wedge-like design of the filter membrane 10′ of FIG. 2, and its substantially similar dimensions to the filter membrane of FIG. 1 are such that its utilization efficiency is also approximately 82%.

As depicted in FIGS. 2A and 2B, the filter membranes of FIG. 1 or FIG. 2, respectively, may each be laid out like tiles along a strip, facing in alternating directions and be cut without cutting wastage from filter membrane strips which have previously been slit by rolling dies to form the shapes outlined above. One of ordinary skill in the art will appreciate that the filter membrane shapes of FIG. 1 or FIG. 2 may be varied, and also that other filter membrane shapes may be used with the present invention, while still enjoying its attendant advantages. Generally, however, the membrane 10 or 10′, regardless of its exact shape, should have a thickness and pore size such that it is able to retain globular solutes having a molecular weight above a threshold, e.g., of at least about 10,000 Daltons. For DNA purification or concentration, the membrane 10 or 10′ preferably should have a pore structure rated to retain globular solutes at least above about 30,000 Daltons, to above about 150,000 Daltons.

Referring now to FIG. 3, a reservoir body 30 or concentration tube of the present invention in which a filter membrane 10 or 10′ of FIGS. 1 or 2 is placed. The reservoir body 30 is generally a tube which has a proximal portion 32 and a distal portion 34 and a longitudinal axis 36. The proximal portion 32 of the tube 30 is cylindrical, e.g., with a

positioned at a height to define a retentate volume 40 of two to twenty microliters.

Referring now to FIG. 4, the filter membrane 10 of FIG. 1 is shown during assembly being placed in the centrifuge tube 30 of FIG. 3. The edges of the filter membrane 10 are curled over so as to fit within the tube 30. Because of its wedge-like[1] design, the membrane is substantially self-guiding. Specifically, once the narrow tip of the membrane 10 is introduced into the tube, and as it is moved through the entry toward the distal, conical portion 34 of the tube 30, the membrane will curl over into a well aligned frustoconical shell which conforms to the shape of the distal, conical portion of the tube, such that its sides 16, 18 come together and its ends 12, 14 each form a complete circumferential edge.

The membrane 10 may be introduced into, and moved distally throughout, the distal portion 34 of the tube with any suitable or appropriately shaped rod, mandrel, fork or the like. The membrane may be introduced into the distal portion 34 of the tube 30 with the same instrument that is to seal the filter membrane as discussed below. Although not specifically shown in FIG. 4, the membrane 10′ of FIG. 2 may be similarly introduced into, and moved distally throughout, the distal portion 34 of the tube 30.

Once it has been moved distally into a predetermined location in the distal portion 34 of the tube 30, the membrane 10, which may optionally be securely maintained in position by applying a vacuum to the outer surface of tube 30 to draw it snugly against ports 38, (or by applying such a vacuum internally of the tip of an insertion mandrel), is then sealed to the tube around its circumference. As shown in FIG. 5, generally there are three bands of sealing of the membrane filter: a proximal seal portion 50, a distal seal portion 52, and at least one vertical edge sealing portion 54.

The filter membrane 10 may be sealed in a number of ways, for example with adhesive or a heat melt polymer in or along the inactive area 22, or by heat fusing with the vessel body in that area. Regardless of the sealing technique, however, the filter membrane 10 should be sealed to the tube 30 around the inactive area 22 such that the filter membrane entirely covers the port area(s) 38, and allows material to exit the tube only through the filter and then through the port(s). Further, the extreme distal end of the filter membrane 10 may extend to and be sealed immediately distal to the port area(s) 38, or it may extend

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substantially square.

The tube 30 of the present invention may be used in any conventional individual or multiple well centrifuge, with such multiple well centrifuges including, but not limited to, rotating platform devices. A plurality of centrifuge tubes 30 (one, two, or as many as needed) of the type shown in FIG. 7 may be separately placed in a multiple well carrier. This carrier may then optionally be placed above a receiver multiwell tray 85 (Figure 10D) used to quantitatively collect filtrate which drips from the bottom point of tip 37 of each tube resting inside the mating receiver well below it. More generally, the tubes 30 may be manufactured as strips or cartridges 80 of eight (see FIG. 8) or twelve tubes, so each strip fills a row or column of a conventional 96-well plateholder. One of ordinary skill in the art, however, will appreciate, that tubes 30 of the present invention may be used in any multiple well centrifuge, regardless of the number of tubes or the matrix orientation of the multiple wells. Furthermore, special adapter plates may be formed, for example, to place four or more such tubes 30 in a larger single well so as to adapt the filtration cell to different existing vessels or centrifuges, or to accommodate a convenient batch size.

Also within the scope of the present invention is an array embodiment, wherein a strip 80 containing a plurality of chambers is made by welding or otherwise bonding a sheet or individual wedges of membrane 10 to two molded halves along the axial plane 75 as shown in Figures 10A-10D, treating or cutting away excess membrane at "e" between the tubes if needed to assure dependable joining or sealing, and then welding together the halves to form an integral strip in which each well half has two vertical sealed edges at 54 formed at or just next to the center plane. In this embodiment, the filter is substantially coextensive with the entire wall of the vessel, so, as shown by the meniscus line 76, essentially the entire chamber surface area participates in filtration.

The tube or tubes 30, 80 of the present invention are shaped so that their placement into an individual well centrifuge, or into the strips or cartridges of a multi-well centrifuge, may be performed by a robotic or another automated device. Similarly, the unobstructed axial position of the retentate well permits addition of sample material and removal of the retentate from the retentate area to be performed by a robotic or other

the filter membrane 10′, so that the 1.0 cm^2 area of this design, which is twofold larger than prior art devices of this volume range, thus results in an exceptionally high ratio of filter membrane active area to fluid volume throughout the course of volume reduction. This is expected to enhance the speed of protein ultrafiltration by a factor of at least two over that of known devices. Further improvement in protein filtration rate, particularly at transmembrane pressures in excess of 150 psi which are obtained when the device of Fig 9 is centrifuged above 12,000 rcf, will result if the inner wall of tube 30 in the region adjacent the active membrane area 20 is molded with a rough textured pattern which provides microchannels for filtrate to flow laterally, along the interstitial space between the membrane and the wall, to the port or ports. The device shown in Figure 10 advantageously has three times the filter area of the embodiment of Figure 9 and a two-thirds greater sample volume capacity, thus further increasing both the throughput and the speed of DNA diafiltration. Further, the cellulosic membrane covers all portions of the plastic chamber walls except the tip, effectively preventing adsorptive loss of DNA to the surface of the polymer vessel wall. In addition, as described above in regard to Figure 9, the filter may be positioned for hydrostatic deadstopping to more quickly reach an endpoint. With this enhanced cycle speed, it becomes efficient to reach a desired degree of purity, for example, to effectively remove PCR primers, by simply performing successive diafiltration cycles. The device thus provides a new and effective method for use in DNA amplification to remove unused primers and harvest the PCR product after amplification.

In this regard, it has been reported (Amicon Publication 304) that optimal retention of PCR DNA product larger than about 500bp and clearance of smaller oligonucleotide primers using YM-100 regenerated cellulose membranes in the Centricon® 100 device requires filtration velocities of no more than one millimeter per minute. The present device of Fig 9 has threefold greater active surface area than any currently available microcentrifuge device that employs the regenerated cellulosic membranes needed for high recovery of DNA, and thus may be expected to result in as much as a threefold reduction in the time required to diafilter DNA at one millimeter per minute.

or to purely conical tubes as mentioned above. In each case, a relatively large filter is primarily supported by solid wall, providing a high ratio of active filter area to reservoir volume, while small ports provide escape for the filtrate.

In providing a concentration vessel wherein the filter is coextensive with a region of the peripheral wall and the retentate is captured in a conical tip, applicant's vessel may be seen to both increase the available area and openness of the filter while allowing effective recovery of extremely small volumes with high efficiency. The ratio of sample volume to retentate volume may be controlled by the relative height of the permeate ports and the provision of increased sample volume in the proximal cylindrical portion of the reservoir. Furthermore, selection of the effective membrane pore size allows a high degree of control over the ultimate percentage recovery and required spin down time.

The vessel of Figure 9 may be implemented in standard sizes identical to those of existing concentration or sedimentation tubes. For example, that device can be configured using a commercial .6 mL microcentrifuge tube to form the retentate reservoir. This size tube can be accommodated in a one-and-a-half to two mL filtrate tube for use with small numbers of samples in a conventional 45° fixed-angle microcentrifuge. Alternatively, the same basic device may be arrayed in 8x12 racks above a 96 well microtiter tray used with a swinging platform rotor. As noted above, the open conical retentate well is suited to robotic sample addition and retentate harvesting using conventional laboratory robotic equipment. Furthermore, with a .6 mL sample volume and a five microliter retentate sump, the concentrator achieves concentration by a factor of over one hundred.

However, scaling up the vessel size encounters several significant limitations if the larger vessels are to be compatible with existing centrifuge equipment. Thus, for example, if a similar tube is to be used with a standard 15 mL sample or sedimentation tube, the reception of filtrate below the retentate sump imposes limits on the size of the microcentrifuge vessel and its contents, whereas if one were to use a 50 mL tube, the lower rotational speeds of the required large capacity centrifuge would significantly limit separation speed of devices using smaller pore size filter membrane to retain molecules in the range of 5,000 to 20,000 Daltons.

rises in the receiving tube during centrifuging.

Figure 12C shows an enlarged sectional view of a preferred implementation of this releasing seal construction in the separation vessel 130 of Figure 12B. As shown, the outer wall 131 of vessel 130 extends upward to a flange 132 that rests upon the body of the receiving vessel 140. The receiving vessel 140 is closed by a cap 141 having a seal gasket 142, and the flange 132 of the separation vessel 130 extends to a top surface 134 which bears against the gasket material 142. This may be a compressible urethane foam gasket material in the cap 141. As shown in the detailed enlarged view of Figure 12C, the upper surface 134 of the separation tube comprises a check seal lip 134a extending upwardly at an inwardly-directed angle against the gasket to form a fluid-tight band closing the top of the separation tube 130 against the gasket. The lip 134a is sufficiently thin and is disposed at an angle so that, as pressurized air is forced up between the outer wall 131 of the separation vessel and the inner wall of the surrounding receiving tube 140, the increasing pressure deflects the lip 134a downwardly, thus allowing air pressure to pass from the vessel 140 into the separation tube 130. For this operation, a molded bypass passage, best seen as passage 212 in Figures 11B and 11C communicates between the surrounding vessel and the space surrounding the lip 134a. As shown in Figure 12A, this seal arrangement allows the separation tube 130 to be overfilled, that is, to be filled to such a high level that when the tube 130 is placed in a tilted rotor it wets a substantial portion of the gasket 142 lying above it, and the lip seals against the relatively high outwardly-directed pressure that initially develops in that area during rotation at high speed before the fluid level drops, without leaking out of the vessel 130 into the receiving tube 140, or leaking outside the cap into the centrifuge drum. The separation tube may therefore be loaded to a 7 mL capacity rather than the lower, angled overflow level of 5.2 mL, thereby achieving 34 % greater effective reservoir capacity and a correspondingly increased concentration ratio of 2333:1. For this purpose, tube 130 is used in cylindrical receiving tube of larger capacity than the existing commercial 15 mL tube of Figure 12A, B. For example, a receiving tube such as tube 240 modified as illustrated in phantom in Figure 11C having a geometry effective to hold 7 mL of filtrate below the port of the vessel 130

separation vessel 230 such as the vessel of Figure 12 illustratively having a seven milliliter capacity. As best seen in the top views, the vessel has four ports 238 equispaced about its circumference, and further includes an alignment guide having the form of a rib or blade 231 that projects radially inward from the vessel wall along a diametral plane. Rib 231 is shown extending from near the mouth (top) of the vessel 230 to a position slightly above the bottom of the ports 238. Further the rib 231, which may, for example, be approximately one-half to two millimeters wide, is positioned in a sector between the ports and extends radially to form an elevated wall that catches and aligns the edges 210a, 210b of the filter membrane 210 (Figure 11D) as it is inserted in the vessel. The membrane 210 preferably is sized or subtends an angle such that the filter edges 210a, 210b butt against the rib on each side of the rib, and the filter 210 bows outwardly in alignment against the vessel wall. As illustrated, the membrane is adhered to the vessel wall along edges 210a, 210b by sealing bands 215a, 215b, respectively, and is further attached at the top and bottom edges 210c, 210d by perimeter sealing bands 215c, 215d. Preferably, the vessel 230 also is formed with a circumferential ledge 234 formed by an indentation of the vessel wall at a height to capture, align and retain the membrane as it is initially inserted into the vessel. That is, the upper edge snaps into position below the ledge 234 after the filter has been inserted down to a level that covers the ports 238. This fully stabilizes and positions the filter, allowing adhesive (if used) to set, or allowing a fusing or welding tool (if used) to be inserted and moved to join the filter to the vessel wall without risk of dislodging or misaligning the filter.

Advantageously the sealing bands 215a-215d occupy relatively little of the filter area; preliminary tests indicate that a band 0.5-0.75 mm wide, and having a net surface area of under one square centimeter will dependably seal a filter of five times that area against the wall of the large vessel of Figures 12A-12Cdiscussed above. Additional sealing bands *a, b, c* as illustrated in phantom in Figure 11D may also be provided to secure the central region of the filter to the vessel wall and prevent ballooning caused by the weight of the filter as the filter area becomes uncovered in approaching the final deadstop volume.

As noted above, the installation of filter membrane in the vessel 130 requires

press the filter against the inner wall of the vessel 230 at the filter's lower edge (region 215d at edge 210d of Figure 11D). Each of the ridges has steep edges and a well defined upper surface, forming a narrow strip of contact area protruding about thirty mils outwardly of the non-contact portion of the thimble surface. Thus, outside the intended weld lines, the thimble surface has a relief effective to avoid heating the non-weld areas of the filter. The ridges 252a, 252b straddle the centering rib 231 (Figure 11A), and may for example constitute a single ridge having a narrow slot to accommodate the rib without contact. When the tool 250 is dropped into the vessel, this slot may orient the tool in the vessel. The various ridges, and bumps at tack line positions, further serve to center the heat transfer tool when it is dropped into the vessel, assuring that when a high level of heat is later applied the intended welds will be uniform and the vessel itself will not rack.

The heat transfer tool 250 has a central bore 255 into which a heat applicator rod 260 is then inserted and advanced down to contact the inner wall of the thimble region 252 of the transfer element 250 and transfer heat into the transfer tool wall along its contact region 253. As noted above, this thimble area of the transfer tool has a thin wall, giving it a minimal thermal mass and rapid heating and cooling characteristics. Heat is then transferred to the surrounding filter. The heating rod 260 contacts the thimble intimately, to uniformly and quickly elevate its temperature, but heating of the filter preferentially occurs at the locations of the protruding ridges or bumps on the external surface of the transfer member 250, which are close to, or bear against, the vessel wall. The tip of heating rod 260 fits precisely in the surrounding transfer tool, and each of the tools 250, 260 is independently held at its upper end to allow precise insertion and removal without binding of the two elements of the heating assembly, and to allow removal of the heating rod 260 without upsetting the bonded filter as it cools and sets.

The transfer tool 250 or its tip region 252 may be preheated or warmed a moderate amount before insertion in the vessel 230. However, in accordance with a principal aspect of this assembly method, the heater rod 260 forms the primary heat source and is superheated, i.e., heated to a temperature which is quite high, e.g., hundreds of degrees higher than the melting point of the vessel material or the filter backing, so that it provides a fast and controlled impulse of heat to bring the transfer element up to a

bonding areas, so water in the filter material weld regions may evolve as steam and has ample space for escaping in the gap between the transfer tool 250 and the inner wall of the vessel 230 and filter. By way of illustration, the heater rod 260, initially at 740-900 °F, may bring the transfer member to about 250-300 °F during the preheating stage for attaching a regenerated cellulose membrane to a polypropylene vessel, and preheating occurs in bonding strips ten to eighty mils wide.

After maintaining the preheating position for several seconds to allow escape of the steam, further pressure or movement is then applied to heater rod 260 or heat sink 240, and the transfer tool and rod advance further into the vessel 260, firmly establishing thermally conductive contact and sealing the filter along the perimeter and other intended weld bands against the wall of the vessel. This welding at full compression is illustrated in Figure 13D. At this point, the transfer tool temperature may illustratively lie in the range of 350-400 °F, while the temperature of the heater rod 260 may have fallen appreciably, e.g., to about 480-600 °F. Following closure of clamps 270 to hold the transfer tool, the heater rod 260 is then withdrawn, allowing the temperature of the vessel to fall by heat conduction into the sink 240 and up the shaft of the transfer member 250 into clamp 270, until the bond has set. In this manner a controlled bolus of heat is preferentially transferred into regions of the delicate filter to weld without abrading or injuring the filter itself. The heat transfer tool is then removed and the finished vessel 230 is withdrawn from the heat sink. As seen in Figure 13F, the bond lines 215a, 215c, 215d, *a, b* are clearly visible in the completed assembly due to fusing together of the vessel wall and backing material in those areas.

This manufacturing method has great advantages in that the thermal mass of the transfer member 250 and the heating rod 260 are precisely determined, and their starting temperatures and the residence time of the heating rod in the heat transfer member 250 may both be set so that precisely controlled amounts of heat are applied to the filter, both for preheating and fusing, while no actual movement of the filter or shearing motions occur when the assembly is at elevated temperature. The heat sink 240 establishes a sharp thermal gradient through the wall of the vessel, allowing the body of the vessel 230 to remain intact and assuring a fast setting time, while high levels of pressure may be

the claims appended hereto and equivalents thereof. All publications and references cited herein are expressly incorporated herein by reference in their entirety.

What is claimed is:

4. The method of claim 3, wherein

i) said closed distal end has a receiving volume less than two percent of the volume of said reservoir body

ii) ratio of area of said filter membrane to volume of said reservoir body is greater than 0.75/cm, and

iii) volume of said reservoir body is between about one-half and two hundred cubic centimeters.

5. The method of claim 3, wherein said closed distal end forms a deadstop having a volume under about 1.0% of the volume of said body.

6. The method of claim 3, wherein said filter forms a truncated cone-shaped active filter area when sealed.

7. The method of claim 6, wherein said filter is sealed along an axial-release direction along narrow band segments that allow filtration through a preponderance of its surface area to said port while being supported by the wall of the reservoir body.

8. An ultrafiltration device, comprising

a hollow, smooth, continuous convex reservoir body having a length, a proximal inlet end, and a closed distal end with a port located in a sloping wall lying in an intermediate region between said inlet end and said closed distal end, and

a filter membrane sealed around the interior of said intermediate region and over said port,

such that

when centrifuged under predetermined conditions, fluid and solutes of a predetermined molecular weight range pass through the filter membrane and exits said port, and

a retentate having a greater predetermined molecular weight accumulates in said closed distal end.

14. The method of claim 11, wherein said closed distal end forms a deadstop having a volume between about 0.04% and 0.3% of the volume of said body.

15. The method of claim 11, wherein the step of sealing is performed in stages to first drive moisture from a sealing region of the filter and then fuse the sealing region and vessel.

16. An ultrafiltration device, comprising

at least one hollow reservoir body, each said body having a length, a proximal inlet, a closed distal end, and a port intermediate its inlet and its distal end, and filter sealed about its perimeter to a wall of said intermediate region and over said port to define between said wall and the filter an interstitial space communicating with the port, such that when centrifuged under predetermined conditions, solvent and solutes having a molecular weight substantially smaller than a predetermined molecular weight pass through the filter and exit the port via said interstitial space, and may optionally be collected in a mating receiving well, while solutes having a molecular weight greater than the predetermined molecular weight accumulate in the closed distal end.

17. A method of forming a centrifugal ultrafiltration device array, such method comprising the steps of

providing two reservoir body halves having multiple chamber regions each chamber region having a port and a portion distal thereto

providing filter sheet to cover at least the port of each chamber region, and

sealing the membrane filter to the reservoir body halves and attaching the body halves to each other so as to form a strip array of chambers wherein the filter sheet entirely covers said ports and the distal portions form closed distal ends of the chambers,

so when the strip array is centrifuged, fluid in the chambers filters through the sheet and passes out the ports such that each chamber is effective to isolate a predetermined amount of a retentate in its closed distal end.

24. A separation system for centrifugal separation of an aliquot of fluid material, such separation system comprising

a centrifuge tube having an elongated inner chamber with a closed bottom end and an open top end

a cap configured for closing the top end

a separation vessel having a filtrate outlet and a closed end for accumulating retentate, the separation vessel being adapted to fit within and effect separation within the centrifuge tube when the centrifuge tube is closed by said cap, such that filtrate passing through the filtered outlet passes to the closed bottom end of the centrifuge tube, and

a relief valve that selectively opens in response to elevated pressure in the centrifuge tube to internally vent pressure from the centrifuge tube into the separation vessel.

25. The separation system of claim 24, wherein said relief valve includes

a deflectable member extending between the cap and the separation vessel, and being operative to deflect under pressure for venting between the separation vessel and the centrifuge tube during centrifuging.

26. The separation system of claim 25, wherein said lip is integrally formed with said separation vessel, and further including a flange having a passage extending therethrough providing pressure communication between interior of said centrifuge tube and a space bounded by said lip.

27. A method of attaching a filter membrane to a separation vessel, such method comprising the steps of

providing the separation vessel as a hamber having a curved interior wall

providing an elevated rib extending along the curved interior wall, and

inserting a shaped piece of filter sheet having side edges into the chamber so that the shaped piece is curled by the wall and a side edge contacts the elevated rib to guide the shaped piece into alignment as it is inserted.

32. A method of fabricating a separation vessel, such method comprising the steps of

providing a vessel wall having a port

positioning a filter within the vessel against the vessel wall over the port

supporting the vessel wall in a heat sink and positioning a heat transfer member against the filter, wherein the heat transfer member includes transfer wall having a first surface and a second surface opposed thereto, the first surface being shaped for selectively bearing against the filter in one or more desired bonding regions, and

contacting the second surface of the heat transfer member with a heated body to heat the transfer member and fuse the filter and vessel wall together in said regions.

33. The method of claim 32, wherein the step of contacting with a heated body is performed by

first contacting the transfer member at a first position or with a first pressure to attain an operating parameter, and

next effecting greater compression for effecting final sealing.

34. The method of claim 33, wherein the step of first contacting is performed for a time effective to selectively drive off steam.

35. The method of claim 34, wherein the step of next effecting greater compression is performed to compress and fuse bond lines fastening the filter.

36. The method of claim 33, further comprising the step of removing the heated body while the filter and vessel wall are stabilized between the transfer member and the heat sink to set the bond regions.

37. The method of claim 33, wherein the transfer member includes a thimble having an external relieved surface to clear the vessel wall and with protruding surface features in said bonding regions.

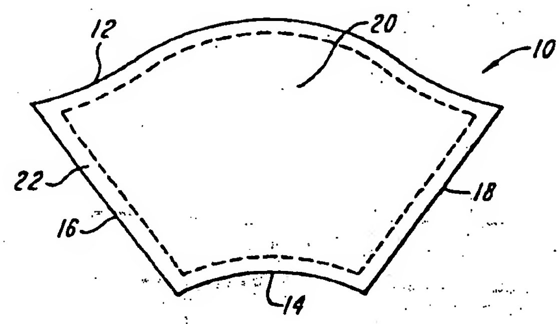

FIG. 1

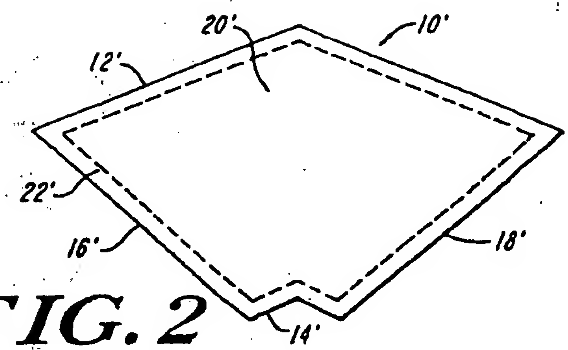

FIG. 2

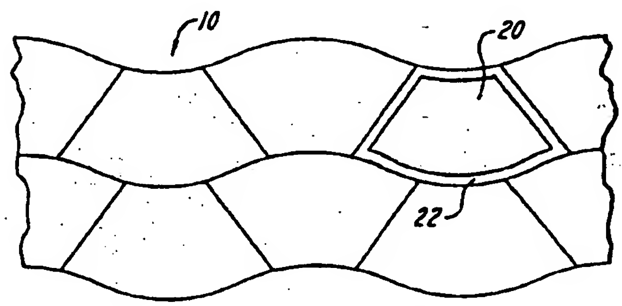

FIG. 2A

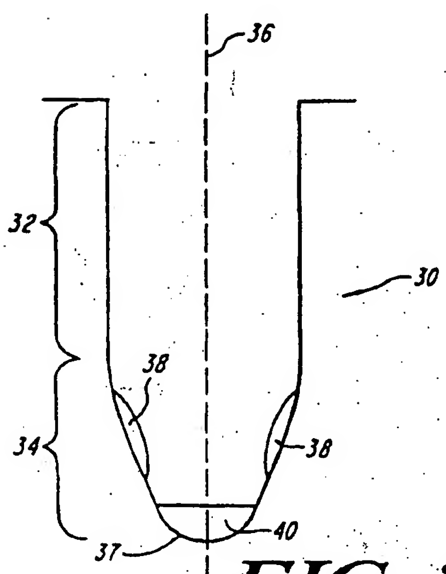

FIG. 3

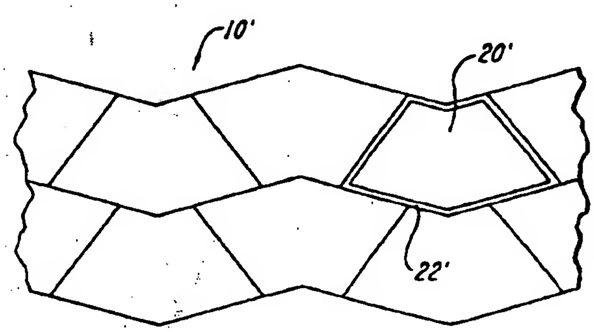

FIG. 2B

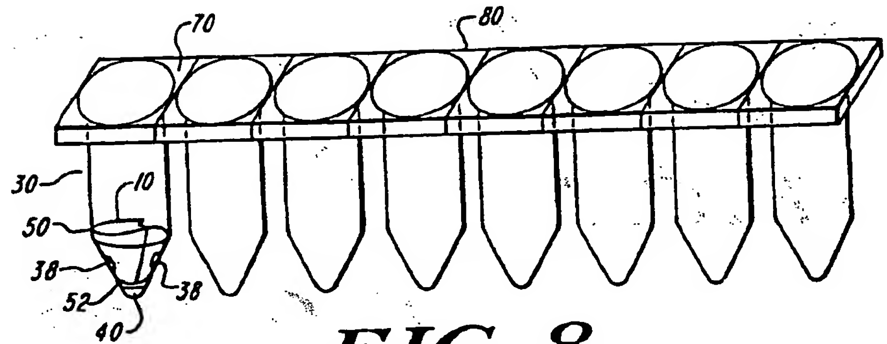

FIG. 8

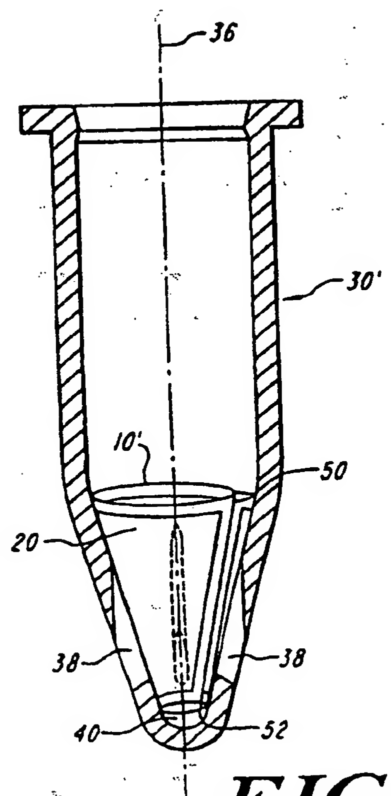

FIG. 9

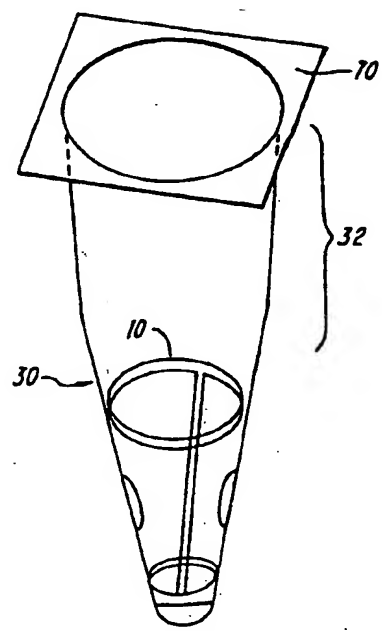

FIG. 7

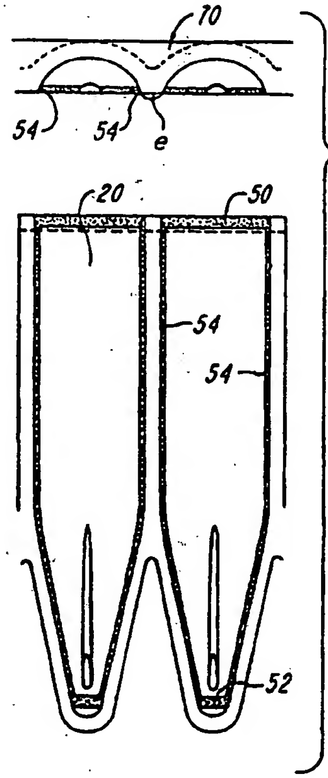

FIG. 10C

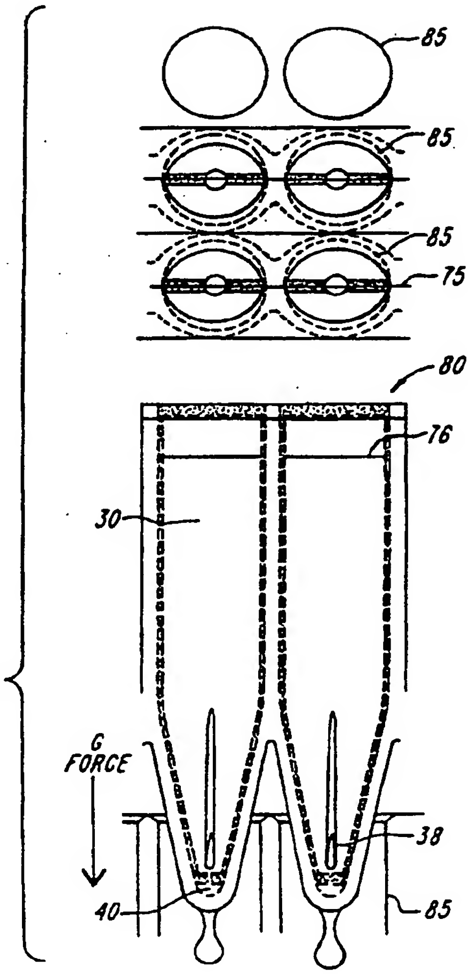

FIG. 10D

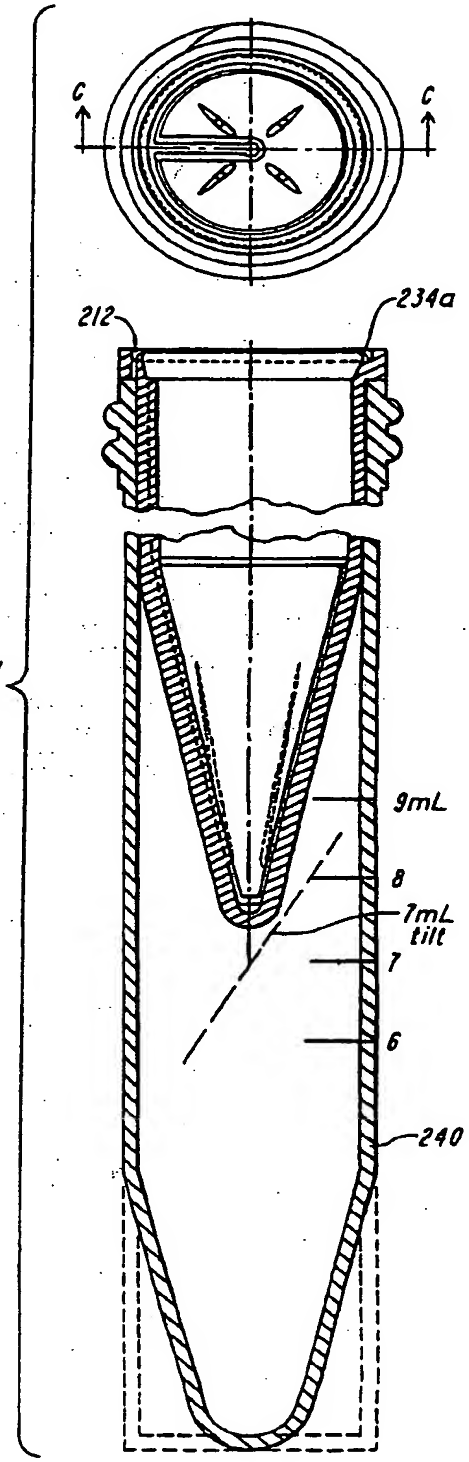

FIG. 11C

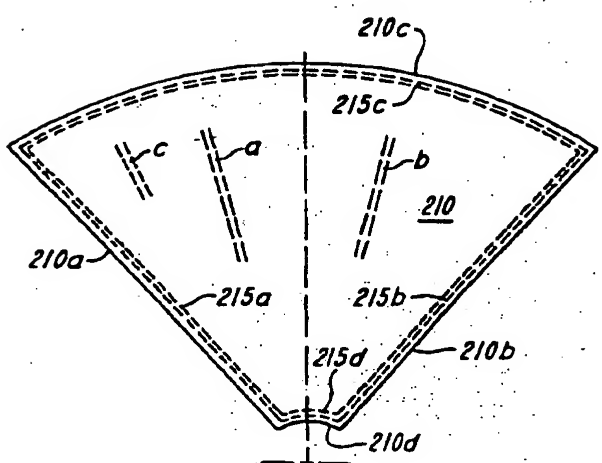

FIG. 11D

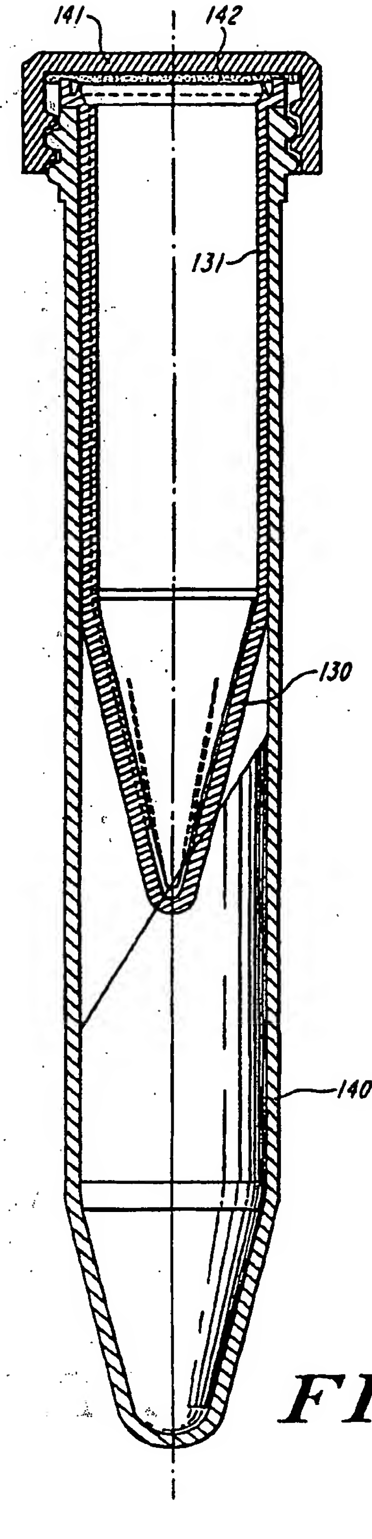

FIG. 12B

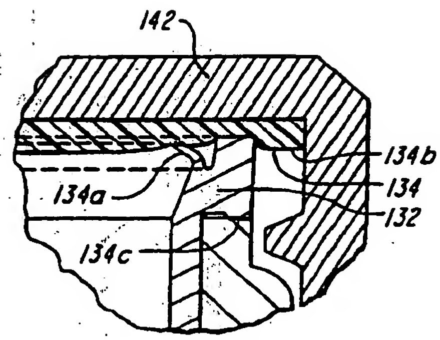

FIG. 12C

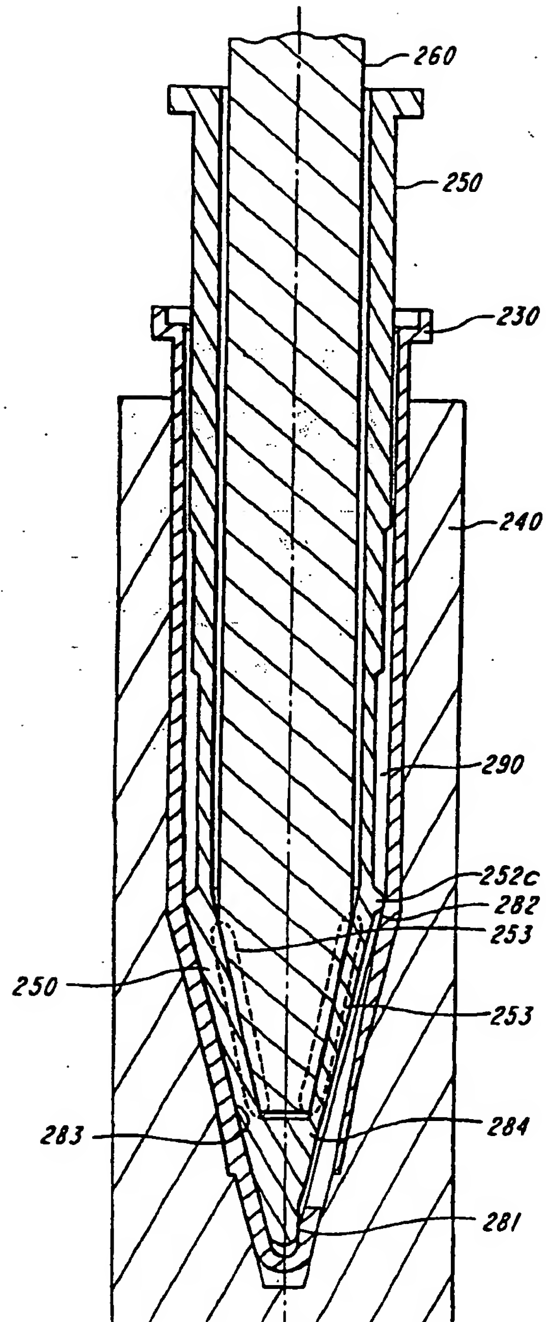

FIG. 13C

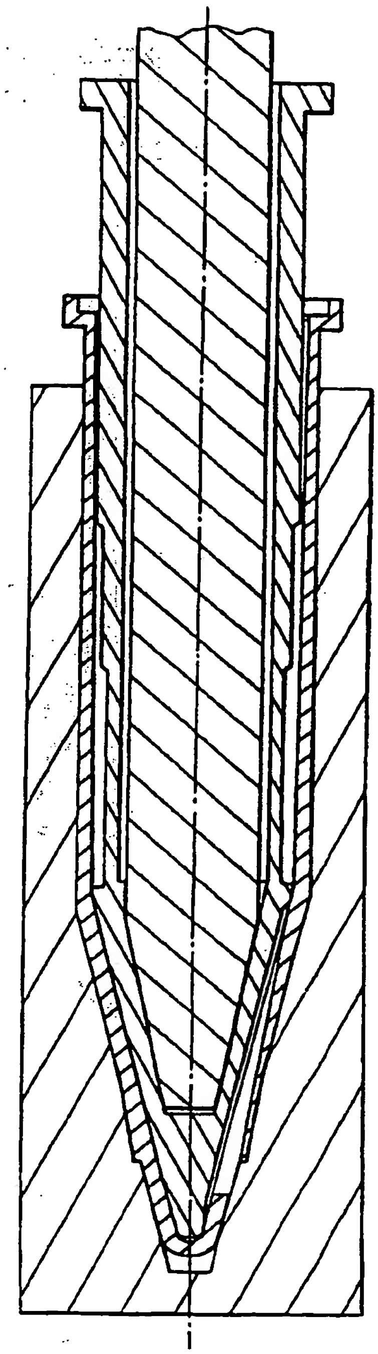

FIG. 13D

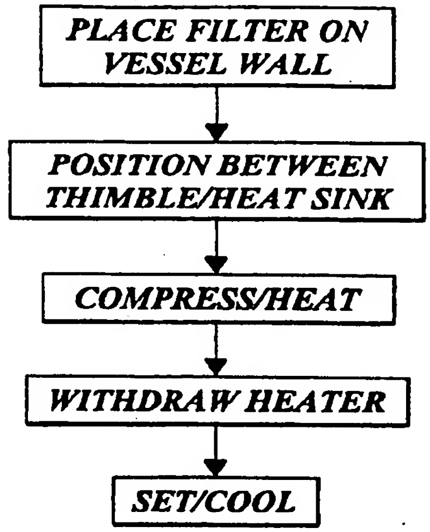
PLACE FILTER ON VESSEL WALL
POSITION BETWEEN THIMBLE/HEAT SINK
COMPRESS/HEAT
WITHDRAW HEATER
SET/COOL

FIG. 14